(19) Japanese Patent Office (JP)

(12) Laid-Open Patent Gazette (A)

(11) Laid-Open Patent Application: No. Sho63-223557

(43) Date of Publication: September 19, 1988

(51) Int. Cl.[4]	Internal Classification	Internal Filing Codes
G 01 N 27/30		F-7363-2G
		J-7363-2G
H 01 L 29/78	301	U-8422-5F

Examination Request: Not requested

Number of Inventions: 1

(Total 4 pages)

---

(54) Title of the Invention: **Method of Manufacture of a Semiconductor Biosensor**

(21) Filing Number: Sho 62-56488

(22) Date of Filing: March 13, 1987

(72) Inventor: Toshihide Kuriyama
c/o NEC Corporation
5-33-1 Shiba, Minato-ku, Tokyo

(71) Applicant: NEC Corporation
5-33-1 Shiba, Minato-ku, Tokyo

(74) Agent: Chieko Tateno, Patent Attorney

Specification

1. Title of the Invention

Method of Manufacture of a Semiconductor Biosensor

2. Claims

(1) In a method of manufacture of semiconductor biosensors consisting of one kind or of two or more kinds of ion-sensitive field effect transistors with an enzyme-immobilized film formed at the surface of a specified sensor part, a method of manufacture of semiconductor biosensors characterized by a process in which the ion-sensitive field effect transistors are formed and moreover a patterned hydrophilic porous film is formed in the sensor area on the semiconductor wafer on which the enzyme-immobilized film is to be provided; by a process in which a solution containing prescribed enzymes is sprayed onto and made to permeate said hydrophilic porous film from an inkjet nozzle so as to form an enzyme film; and by a process in which the enzyme in this enzyme film is immobilized.

(2) A method of manufacture of Claim 1 of the present invention, wherein the semiconductor biosensor is a semiconductor multibiosensor formed by integration of ion-sensitive field effect transistors with a plurality of mutually differing enzyme-immobilized films formed on the specified sensor part surface.

3. Detailed Description of the Invention

[Industrial Field of the Invention]

The present invention concerns a method for manufacture of semiconductor biosensors, and in particular a method of manufacture of semiconductor biosensors consisting of one kind or of two or more kinds of ion-sensitive field effect transistors with an enzyme-immobilized film provided on the surface.

[Prior Art]

As one kind of biosensor which measures the concentration of a specific organic substance in solution, the ion sensitive field effect transistor (hereafter "ISFET"), with an enzyme-immobilized film on its surface, has been known (B. Danielson, I. Lundström, K. Mosbach and L. Stiblert, "On a new enzyme transducer combination: the enzyme transistor," Anal. Lett., 12 (B11), pp. 1189-1199 (1979)). By using the ISFET to detect changes in the concentration of hydrogen ions in the film on decomposition, by catalytic action of the enzymes of the enzyme-immobilized film, of a specific organic substance in solution, this ISFET biosensor measures the concentration of a specific organic substance. As examples of enzyme-immobilized films with such selectivity, urease-immobilized film is known as a means of detecting urea, and glucose oxidase-immobilized film is known as a means of detecting glucose.

Further, multi-biosensors capable of simultaneous measurement of numerous organic substances in a solution can also be realized by providing, on the surfaces of the respective prescribed ISFETs, a plurality of enzyme-immobilized films (Toshihide

Kuriyama, Jun Kimura and Mie Kawana: "Integrated SOS/ISFET multi-biosensors," Trans. IECEJ, Tech. Rep.[1] Electronic Devices, ED84-158, p. 19 (1984)).

[Problems Which the Present Invention Attempts to Solve]

However, in the aforementioned semiconductor biosensors, in order to form the enzyme-immobilized film an enzyme solution was dropped onto the sensor region by a person using a syringe; because of this, it was difficult to control the thickness and shape of the enzyme-immobilized film. In recent years, a method has been reported in which an inkjet nozzle is used to drop the enzyme solution onto the sensor area surrounded by film resist (Mie Kawana, Jun Kimura and Toshihide Kuriyama: "Semiconductor multi-biosensors and their applications," Preprints Fall Conf. Electrochemistry '85, D311 (1985)).

However, though the planar shape of the enzyme-immobilized film can be controlled in this case, the film thickness tended not to be uniform. This is because the enzyme solution adhering to the wafer surface dries from the periphery, so that the enzyme-immobilized film becomes thick near the periphery.

The purpose of the present invention is to eliminate this defect of the Prior Art, and in particular to provide a method of manufacture of semiconductor biosensors enabling the formation at the wafer stage, at a prescribed position on the surfaces of a plurality of ISFETs formed on a single chip, of miniature biosensors with excellent productivity and excellent uniformity of characteristics.

[Measures to Solve the Problem]

In a method for the manufacture of a semiconductor biosensor consisting of one kind or of two or more kinds of ion-sensitive field effect transistors in which enzyme-immobilized film is formed at the surface of a specified sensor part, the present invention is a method for the manufacture of semiconductor biosensors, characterized by a process in which the ion-sensitive field effect transistors are formed and moreover a patterned hydrophilic porous film is formed in the sensor area on the semiconductor wafer on which the enzyme-immobilized film is to be provided; by a process in which a solution containing prescribed enzymes is sprayed onto and made to permeate said hydrophilic porous film from an inkjet nozzle so as to form an enzyme film; and by a process in which the enzyme in this enzyme film is immobilized.

In the present invention, either inorganic material or organic material may be used as the hydrophilic porous film, so long as it is material enabling permeation and retention of the enzyme-containing solution in the porous material. Methods for immobilizing enzymes in the enzyme film include the method of adding a bridging agent to an enzyme

---

[1] [translator's note] This society, currently known as IEICE (Institute of Electronics, Information and Communication Engineers), has long published a series of technical articles called "Technical Reports" (Japanese *gijutsu kenkyuu houkoku*) in a series of fields. The naming convention is that used here, i.e. the English initials of the field followed by two numbers separated by a hyphen. I have therefore assumed that the Japanese title appearing here, *kenkyuukai shiryou*, is the title in use in 1984, and that this was subsequently changed to *gijutsu kenkyuu houkoku*.

film, and adding an optical bridging agent to an enzyme-containing solution, using this solution to form an enzyme film, and then inducing photo-crosslinking. The present invention is especially useful when the semiconductor biosensor is a semiconductor multi-biosensor formed by integrating a plurality of ion-sensitive field effect transistors, each with mutually different enzyme-immobilized films formed on the prescribed sensor part surface.

[Operation]

In the present invention, an enzyme-containing solution sprayed using an inkjet nozzle adheres to a patterned hydrophilic porous film, and then permeates the film. The enzyme solution is retained within said hydrophilic porous film by means of surface tension, is spread essentially uniformly, and after drying as well the enzyme is distributed uniformly. Hence by using a patterned hydrophilic porous film of uniform thickness, a patterned enzyme-immobilized film of uniform thickness is obtained. Further, by placing a wafer on an X-Y stage and moving to a precise position, the enzyme may be dropped onto a prescribed sensor area; moreover, by controlling the amount of enzyme that is dropped, enzyme-immobilized films with similar characteristics can be formed on a wafer.

[Working Example]

Below a Working Example of the present invention is explained in detail, referring to the drawings.

Figure 1 and Figure 2 are used to explain one Working Example of the method of manufacture of semiconductor biosensors of the present invention. Figure 1 is a summary diagram showing the process of formation of enzyme-immobilized film on a semiconductor wafer on which are formed ion-sensitive field effect transistors (ISFETs); Figure 2 is a summary plane diagram of a semiconductor wafer on which are provided patterned hydrophilic porous films. In this Working Example, the case of formation of an urease-immobilized film on the ISFET sensor part for detection of urea is explained as an example.

In the Working Example using inorganic material as the patterned hydrophilic porous film 2 formed on the semiconductor wafer 1, a ceramic material containing alumina powder and polyvinyl alcohol was printed at a prescribed position on the semiconductor wafer 1 by a screen printing method, and was then sintered at high temperature; by this means the polyvinyl alcohol evaporated, and a patterned hydrophilic porous film 2 was formed. Here the thickness of the hydrophilic porous film 2 could be controlled through the viscosity of the ceramic material and the screen thickness. On the other hand, in a Working Example using an organic material as the hydrophilic porous film 2, photosensitive polyvinyl alcohol resin with carbonate (in the present Working Example, calcium carbonate) added was used, and after application to the semiconductor wafer 1, photolithographic techniques were used to form a patterned film. The carbonate was then evaporated at high temperature to form the hydrophilic porous film 2. Next a solution of urease and bovine serum albumin dissolved in tris hydrochloric acid buffer solution was inserted into the ink container 3b of the inkjet 3, and by applying a voltage pulse of approximately 20 V to the piezoelectric element of the inkjet nozzle 3a, droplets

4 containing urease were sprayed from the inkjet nozzle onto the patterned hydrophilic porous film 2. The size of the droplets 4 could easily be specified by the size of the nozzle; in the present Working Example, droplets with diameters ranging from 20 to 100 μm were used. Moreover, the solution of urease and bovine serum albumin was diluted with tris hydrochloric acid buffer solution (pH 8.5) to lower the viscosity. In forming the urease immobilized film, the number of droplets dropped onto the aforementioned patterned hydrophilic porous film could be controlled and specified accurately through the voltage pulses.

Next a solution containing a bridging agent (in the present Working Example, glutaraldehyde) was sprayed by the inkjet method onto the aforementioned patterned hydrophilic porous film as droplets, similarly to the aforementioned solution containing urease. By causing this to react with the urease, it was possible to manufacture a semiconductor biosensor in which a urease-immobilized film is formed on the prescribed sensor part of the ISFET. As the means of immobilizing the enzyme, in addition to the aforementioned method of adding a bridging agent later in the process, an immobilization method may also be adopted in which an optical bridging polymer is dissolved in the enzyme in advance, and after introducing this solution into the patterned hydrophilic porous film, light is made incident to cause immobilization.

Further, by repeating the aforementioned process for different enzymes, it was possible to manufacture a semiconductor multi-biosensor in which are integrated a plurality of ISFETs with different enzyme-immobilized films on the surface.

Figure 3 and Figure 4 are biosensor cross-sectional diagrams used to explain a Working Example in which the method of the present invention was employed in manufacture of a semiconductor multi-biosensor formed on a silicon layer with an island morphology provided on a sapphire substrate. Figure 3 is a cross-sectional diagram of a sensor after formation of the hydrophilic porous film; Figure 4 is a cross-sectional diagram of a sensor after formation of the enzyme-immobilized film. In both figures, 12 is the hydrophilic porous film, 5 is the sapphire substrate, 6 is n^+ type silicon with a high concentration of impurities, 7 is p type silicon, 8 is a silicon oxide film, 9 is a silicon nitride film, and 10a, 10b and 10c are each enzyme-immobilized films formed using different kinds of enzymes. As shown in Figure 4, a plurality of enzyme-immobilized films are formed on a single chip; by using this sensor, it is possible to simultaneously detect a plurality of substrates in a sample.

On measuring the scattering in sensitivity of approximately 800 semiconductor multi-biosensors manufactured by the method of the present invention on a semiconductor wafer (diameter 4 inches), the scattering was within 10% and within 5% for urea sensors employing urease-immobilized film and for glucose sensors employing glucose oxidase-immobilized film respectively. These values are equal or superior to the precision of biosensors using individual enzyme-immobilized films.

[Effect of Invention]

As explained above, by means of the present invention it is possible to conveniently and precisely control the amount of enzyme adhering to the ISFET surface through the voltage pulses applied to the piezoelectric element of the inkjet nozzle, and in

addition the region in which the enzyme-immobilized film is provided is regulated by the patterned hydrophilic porous film, so that both the area and the thickness of the enzyme-immobilized film can be precisely controlled.

Because of the aforementioned advantages, the method of the present invention enables the manufacture with good productivity of biosensors with uniform characteristics. In the manufacture of semiconductor multi-biosensors in particular, the method is especially advantageous for its improvement of the precision of the film thickness, which had previously been difficult.

4. Brief Explanation of the Drawings

Figure 1 is a summary diagram showing the process of enzyme-immobilized film formation in one Working Example of the method of the present invention; Figure 2 is a summary plane diagram of a semiconductor wafer provided with patterned hydrophilic porous films; Figure 3 is a cross-sectional diagram of sensors after formation of hydrophilic porous films on island-morphology silicon on a sapphire substrate by the method of the present invention; and Figure 4 is a cross-sectional diagram of sensors after formation of enzyme-immobilized films on the same island-morphology silicon.

1: Semiconductor wafer

2, 12: Hydrophilic porous film

3: Inkjet

3a: Inkjet nozzle

3b: Ink container

4: Droplet

5: Sapphire substrate

6: n^+ type silicon

7: p type silicon

8: Silicon oxide film

9: Silicon nitride film

10a, 10b, 10c: Enzyme-immobilized film

Patent Agent Chieko Tateno

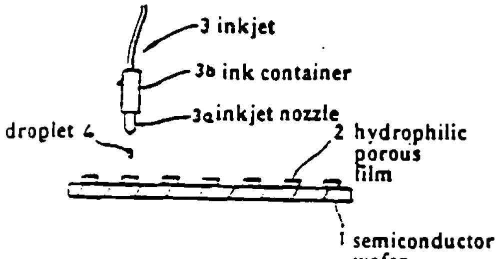

Figure 1

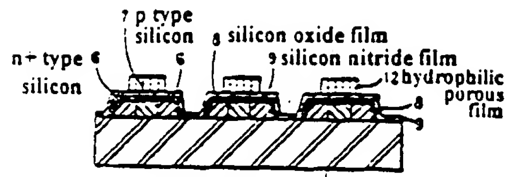

Figure 3

Figure 2

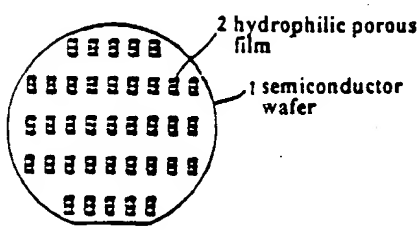

Figure 4